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Erysiphe acantholimonis sp. nov. on *Acantholimon hedinii*

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Abstract—A powdery mildew on *Acantholimon hedinii* was collected in China, examined, described, and proposed as a new species, *Erysiphe acantholimonis*. Recognition of this new species is supported by phylogenetic analyses of the internal transcribed spacer regions including 5.8S and 28S rDNA sequences from three *E. acantholimonis* specimens. A comparison with and a key to the three species of *Erysiphe* on hosts belonging to the family *Plumbaginaceae* are provided.

Key words—*Erysiphaceae, Erysiphe aurea, Erysiphe limonii,* ITS, molecular phylogeny

Introduction

Powdery mildews (*Erysiphaceae*) are holobiotrophic fungi pathogenic on plant leaves, stems, flowers, and fruits. These pathogens cause moderate to serious damage in nearly 10,000 species of angiosperms (Amano 1986, Glawe 2008). Braun (1987) introduced a classification of the *Erysiphaceae* that split the family into 18 genera, including *Erysiphe*, *Microsphaera*, and *Uncinula.* On the basis of phylogenetic analyses, Braun & Takamatsu (2000) subsequently reduced these three genera to synonymy under *Erysiphe* (sensu lato), which they separated into three non-phylogenetic sections: *E.* sect. *Erysiphe, E.* sect. *Microsphaera,* and *E.* sect. *Uncinula. Erysiphe* sect. *Erysiphe* is characterized by having chasmothecia with mycelioid appendages.

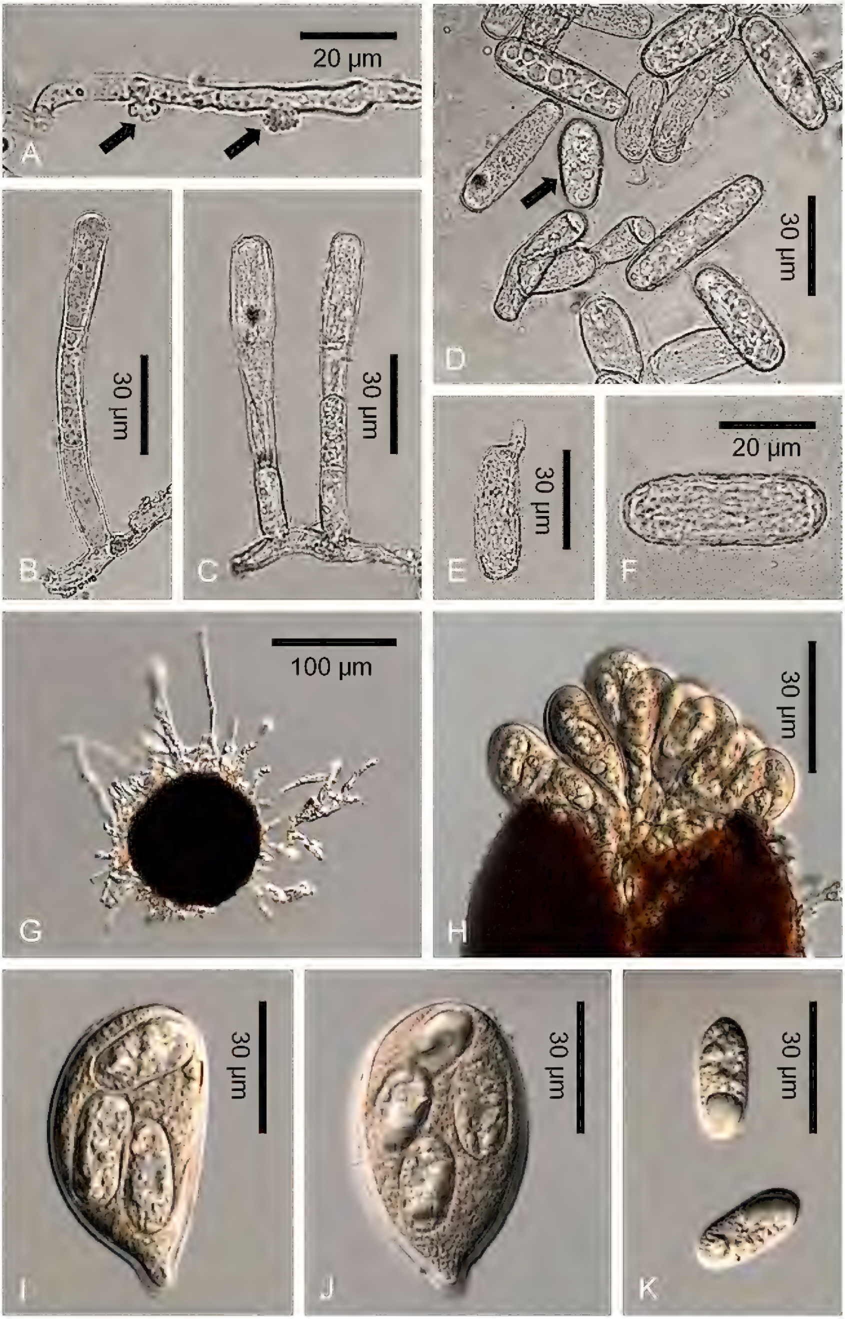
Two species of *Erysiphe* sect. *Erysiphe*, *E. aurea* R.Y. Zheng & G.Q. Chen and *E. limonii* L. Junell, have been described on plants within the family *Plumbaginaceae*. In 2010, a powdery mildew occurring on *Acantholimon* (*Plumbaginaceae*) was found in Xinjiang, China, which Song et al. (2015) reported as the first record of *Erysiphe limonii* on *A. hedinii* from China. However, a detailed morphological re-examination and molecular analyses have revealed that powdery mildew on this host is clearly distinct from *E. limonii*. Here, we propose the fungus collected on *A. hedinii* as a new species, *E. acantholimonis,* and base our description on a morphological characterization and DNA sequence comparison. In addition, we discuss relationships of the new species to closely related powdery mildew fungi.

Materials & methods

Fungal structures were detached from the infected leaves and mounted in a few drops of distilled water on a glass slide for light microscopy. Morphological characteristics of the fungal structures were examined in bright field and differential interference contrast (DIC) light microscopy, using an Olympus BX51 microscope (Olympus, Tokyo, Japan) for measurements and a Zeiss AX10 microscope equipped with AxioCam MRc5 (Carl Zeiss, Göttingen, Germany) for photographs. Elements were measured at 100×, 200×, 400×, and 1000× magnifications. Representative voucher specimens were deposited in the Mycological Herbarium of Tarim University, Xinjiang, China (HMUT), and the Korea University Herbarium, Seoul, Korea (KUS).

Genomic DNA was extracted from chasmothecia using the Chelex 100 method as set forth in Walsh et al. (1991) and Hirata & Takamatsu (1996). The 28S ribosomal DNA (rDNA) including domains D1 and D2 and the internal transcribed spacer (ITS) regions including ITS1, 5.8S, and ITS2 were amplified by polymerase chain reaction (PCR) using the primer pairs ITS1/ITS4 (White et al. 1990) for ITS, and PM3 (Takamatsu & Kano 2001) and TW14 (Mori et al. 2000) for 28S. The PCR products were then sequenced directly (Takamatsu et al. 2009) by a commercial sequencing company (Macrogen, Seoul, Korea) using the same primers used for PCR. The resulting sequences were deposited in GenBank (Accession Nos. KU554532–KU554537). The sequences were used in a BLASTn search against the NCBI nucleotide databases to confirm the identity of the isolates. The sequences were aligned with published sequences of *E.* sect. *Erysiphe* species retrieved from GenBank, using the online version of MAFFT ver. 7.215 (Katoh et al. 2002). *Neoerysiphe galeopsidis* (DC.) U. Braun was used as an outgroup. Maximum Likelihood (ML) analyses were performed using RAxML-HPC BlackBox ver. 8.2.4 (Stamatakis 2006, Stamatakis et al. 2008) in the CIPRES cluster server (http://www.phylo.org/) at the San Diego Supercomputer Center.

Fig. 1. *Erysiphe acantholimonis* (holotype, HMUT00821). A: hypha with appressoria (arrows); B, C: conidiophores; D: conidia (arrow indicates a primary conidium); E: germinating conidium; F: surface structure of a wrinkled conidium; G: chasmothecium with appendages; H: chasmothecium containing several asci; I, J: asci containing 3–4 ascospores; K: ascospores.


20 µm
A
30 µm
30 µm
B
C
30 µm
D
30 µm
E
20 µm
F
100 µm
G
30 µm
H
30 µm
I
30 µm
J
30 µm
K

Taxonomy

Erysiphe acantholimonis J.G. Song, B. Xu & H.D. Shin, **sp. nov.** FIG. 1

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Differs from *Erysiphe limonii* by its larger conidial length/width ratio, its smaller 2–4-spored asci, and its *Acantholimon* host.

TYPE: China, Xinjiang, Wuqia county, 39°53′17″N 74°56′47″E, on *Acantholimon hedinii* Ostenf. (*Plumbaginaceae*), 29 Aug. 2010, B. Xu (**holotype**, HMUT00821; **isotype**, KUS-F29047; GenBank KU554537, KU554536).

ETYMOLOGY: The epithet is derived from the host genus, *Acantholimon*.

COLONIES amphigenous, thinly effused or conspicuous, forming circular to irregular white patches. HYPHAE straight or almost so, branched, hyaline, septate, 4–8 µm diam. HYPHAL APPRESSORIA well-developed, moderately lobed to multi-lobed, solitary or in opposite pairs, 4–9 µm diam. CONIDIOPHORES arising from the upper part of mother cells, 72–105 × 6.5–9.0 µm, producing conidia singly. FOOT-CELLS mostly straight or almost so, cylindrical, 17–38 µm long, relatively short, followed by 2–3 shorter cells. CONIDIA hyaline, cylindrical-oval to oblong, 30–50 × 11–15 µm with a length/width ratio of 2.0–4.1, lacking fibrosin bodies, producing germ tubes in a perihilar position, with an angular/rectangular wrinkling pattern on the surface. PRIMARY CONIDIA apically rounded, basally subtruncate, generally smaller than the secondary conidia. CHASMOTHECIA amphigenous, cauligenous, gregarious, dark brown, spherical, 80–130 µm diam, containing 5–10 asci. PERIDIUM CELLS irregularly polygonal or rounded, 10–20 µm diam, inner wall of the peridium cells yellowish to hyaline. APPENDAGES few to numerous, 0–1-septate, mycelioid, 0.5–1 times as long as the chasmothecial diameter, 50–100 × 7.5–9.0 µm, tips distinctly recurved, brown at the base, becoming paler toward the tip. ASCI obovoid to ellipsoid or saccate, mostly short-stalked, 52–68 × 32–40 µm, containing 2–4 spores per ascus. ASCOSPORES oblong-ovoid, hyaline, guttulate, 25–32 × 12–15 µm.

ADDITIONAL SPECIMENS EXAMINED: **on *Acantholimon hedinii*: CHINA, XINJIANG, Wuqia county,** 19 Jun. 2010, B. Xu (HMUT00507, KUS-F29044); 28 Aug. 2010, B. Xu (HMUT01489, KUS-F29046; GenBank KF318724, KU554532); **Wensu county,** 24 Aug. 2010, B. Xu (HMUT00396, KUS-F29045; GenBank KU554535, KU554534).

HOST RANGE AND DISTRIBUTION: on *Acantholimon hedinii* (China, endemic).

Phylogenetic analyses

ADDITIONAL SPECIMEN SEQUENCED: ***Erysiphe limonii*: KOREA, IKSAN,** 11 May 2013, H.D. Shin (KUS-F27301; GenBank KU554533).

The GenBank BLAST search of the examined powdery mildew isolates showed that *E. acantholimonis* on *A. hedinii* belongs to *E.* sect. *Erysiphe*. Based on the

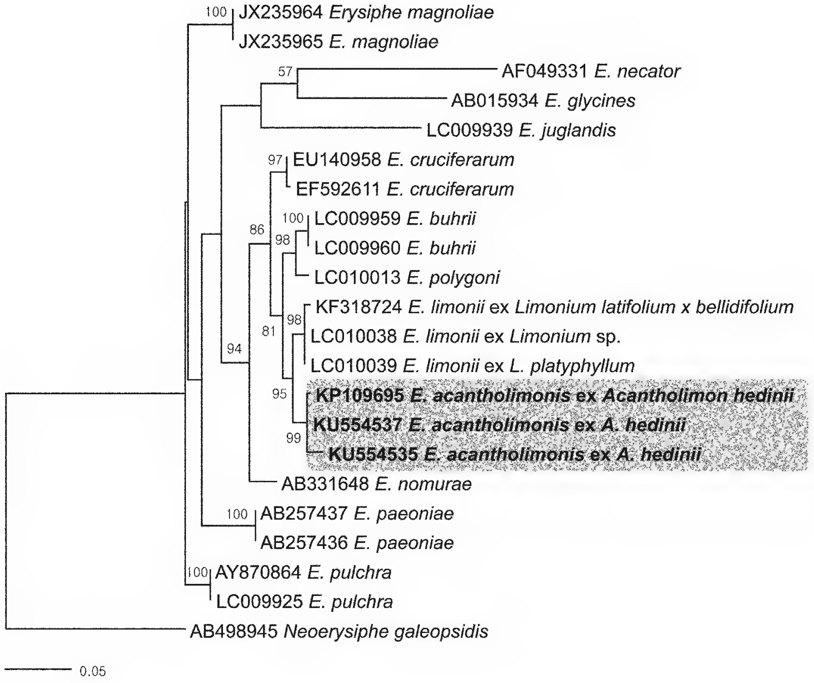
100
JX235964 Erysiphe magnoliae
JX235965 E. magnoliae
57
AF049331 E. necator
AB015934 E. glycines
LC009939 E. juglandis
97
EU140958 E. cruciferarum
EF592611 E. cruciferarum
100
LC009959 E. buhrii
86
98
LC009960 E. buhrii
LC010013 E. polygoni
KF318724 E. limonii ex Limonium latifolium x bellidifolium
98
81
LC010038 E. limonii ex Limonium sp.
94
LC010039 E. limonii ex L. platyphyllum
95
KP109695 E. acantholimonis ex Acantholimon hedinii
KU554537 E. acantholimonis ex A. hedinii
99
KU554535 E. acantholimonis ex A. hedinii
AB331648 E. nomurae
100
AB257437 E. paeoniae
AB257436 E. paeoniae
100
AY870864 E. pulchra
LC009925 E. pulchra
AB498945 Neoerysiphe galeopsidis
0.05

Fig. 2. ITS (including 5.8S rDNA) phylogenetic relationship between *Erysiphe acantholimonis* isolates and reference isolates retrieved from GenBank, inferred by RAxML analysis. Sequences of *E. acantholimonis* are shown in bold and their clade is highlighted in gray. Numbers above the branches represent bootstrap support values >50% obtained from 1000 bootstrap replicates. Scale bar indicates the number of substitutions per site.

molecular phylogenetic trees generated from the RAxML analyses of the ITS and 28S rDNA, sequences generated from the three *E. acantholimonis* isolates formed an independent clade with bootstrap (BS) supports of 99% for ITS (Fig. 2) and 86% for 28S (Fig. 3). The clade of the new fungus was clearly separated from *E. limonii* from Korea (KF318724 and KU554533 ex *Limonium latifolium* × *bellidifolium*) and Ukraine (LC010038 ex *Limonium* sp.; and LC010039 ex *L. platyphyllum*) in the RAxML trees. In addition, *E. acantholimonis* was shown to be a sister species to *E. limonii* with BS supports of 95% for ITS (Fig. 1) and 98% for 28S (Fig. 3), suggesting a close phylogenetic relationship. Thus the status of *E. acantholimonis* as a new species is fully supported by the molecular sequence analyses.

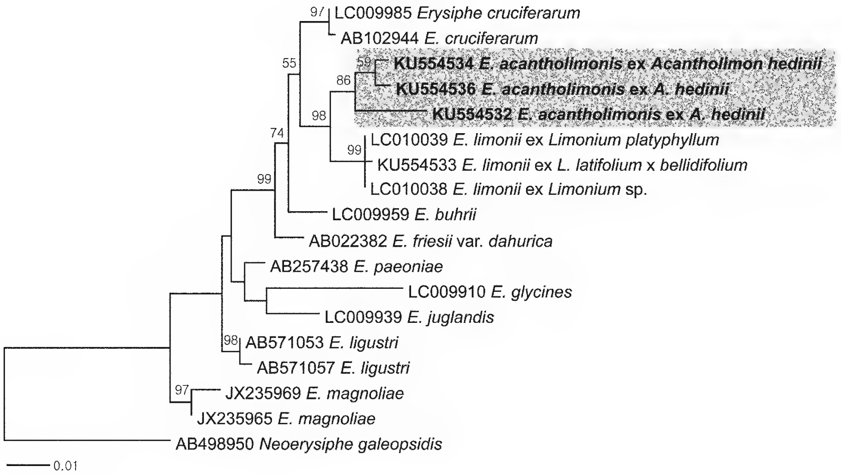
97
LC009985 Erysiphe cruciferarum
AB102944 E. cruciferarum
55
59
KU554534 E. acantholimonis ex Acantholimon hedinii
86
KU554536 E. acantholimonis ex A. hedinii
98
KU554532 E. acantholimonis ex A. hedinii
74
99
LC010039 E. limonii ex Limonium platyphyllum
KU554533 E. limonii ex L. latifolium x bellidifolium
99
LC010038 E. limonii ex Limonium sp.
LC009959 E. buhrii
AB022382 E. friesii var. dahurica
AB257438 E. paeoniae
LC009910 E. glycines
LC009939 E. juglandis
98
AB571053 E. ligustri
AB571057 E. ligustri
97
JX235969 E. magnoliae
JX235965 E. magnoliae
AB498950 Neoerysiphe galeopsidis
0.01

Fig. 3. 28S rDNA phylogenetic relationships between *Erysiphe acantholimonis* isolates and reference isolates retrieved from GenBank, inferred by RAxML analysis. Sequences of *E. acantholimonis* are shown in bold and their clade is highlighted in gray. Numbers above the branches represent bootstrap support values >50% obtained from 1000 bootstrap replicates. Scale bar indicates the number of substitutions per site.

Discussion

Two *Erysiphe* species belonging to *E.* sect. *Erysiphe* have previously been recorded as occurring on *Plumbaginaceae* worldwide (Braun & Cook 2012, Farr & Rossman 2016). *Erysiphe limonii*, found from Europe to Asia on various *Limonium* spp., is morphologically similar to *E. aurea* but differs by having appendages with some septa, 3–8 asci, and an inner peridial wall that is not golden yellow (Braun & Cook 2012). The phylogenetic status of *E. limonii* was recently resolved (Takamatsu et al. 2015, Choi et al. 2016), resulting in a characterization based on morphological observations and a molecular sequence analysis. *Erysiphe aurea* was described from a single collection on *Limonium suffruticosum* in China (Zheng & Chen 1980, Braun & Cook 2012); it is distinguished from *E. limonii* in having aseptate appendages and an inner peridial wall composed of golden-yellow cells. The conidial state of *E. aurea* is inadequately known, and molecular data are not yet available for phylogenetic analyses. Therefore, additional material is needed to ascertain the taxonomic status of *E. aurea*, and to clarify its relationship to *E. limonii* and *E. acantholimonis.*

Erysiphe limonii differs from *E. acantholimonis* by its larger, 3–6-spored asci (45–85 × 25–50 µm) and smaller conidial length/width ratio (1.6–2.9; Choi et al. 2016), while *E. aurea* differs from *E. acantholimonis* by its longer and narrower (60–80 × 25–35 µm) 4–6-spored asci and golden yellow inner peridial wall.

Acantholimon comprises about 200 species worldwide (Dogan & Akaydin 2004). Recent molecular phylogenetic studies (Lledó et al. 2005, Moharrek et al. 2014) have shown that *Limonium* and *Acantholimon* are not closely related, suggesting that the *Acantholimon* and *Limonium* powdery mildews are unlikely to be accepted as conspecific. Amano (1986) listed *Acantholimon avenaceum*, *A. kotschyi*, and *Acantholimon* sp. as hosts of *E. limonii*. Additional morphological and molecular data are needed to supplement our current knowledge about powdery mildews infecting *Acantholimon* spp.

DNA sequence comparisons based on the ITS and 28S rDNA data obtained from the analyzed *Acantholimon* specimens place their powdery mildew fungus in *E.* sect. *Erysiphe*. In addition, the results indicate that *E. limonii* and *E. acantholimonis* may have diverged along with their plumbaginaceous hosts. Results obtained from the phylogenetic analyses are further supported by morphological data, showing that *E. acantholimonis* is a distinct species occurring on *A. hedinii* in China. Based on the information obtained from literature as well as our own data, we provide a key to the three species of *Erysiphe* occurring on *Plumbaginaceae*:

Key to *Erysiphe* species known on *Plumbaginaceae*

1. Inner peridial wall golden yellow; appendages aseptate .... ***E. aurea***
1. Inner peridial wall not golden yellow; appendages at least partly septate 2
2. Asci relatively large (45–85 × 25–50 µm), 3–6-spored;
    median conidial length/width ratio <3.0 .... ***E. limonii***
2. Asci relatively small (52–68 × 32–40 µm), 2–4-spored;
    median conidial length/width ratio mostly >3.0 .... ***E. acantholimonis***

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